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Review of bachelor thesis

Title: „ BILIRUBIN ESTERIFICATION USING ALIPHATIC ALCOHOLS "

Author: Kristýna Poslední

The Bachelor Thesis entitled "Bilirubin esterification using aliphatic alcohols" by Ms Kristyna Poslední deals with the chemical modification of bilirubin by adding to its chemical structure *n*-alcohols with a number of carbons ranging from 1 to 8. The chemically-modified bilirubin is intended to be used as a model compound to study its ability to form self-aggregates or how it can interact with other pigments forming aggregates. In this study efforts were made to optimize experimental conditions to increase the yield of the esterification reaction under acidic conditions. To reach this goal, suitable solvents, optimal temperature, ratios between the solvents and the *n*-alcohols and variations in the percentage of acid in the reaction medium were thoroughly investigated. Additionally, the chemical products and yields of the reactions were analyzed by HPLC, but before that the student also investigated what were the optimal stationary phase, the length of the column and composition of the mobile phase to better separate the products of the esterification reaction.

In overall, the Bachelor Thesis is clearly written in English, uses a direct language and the results and discussion are well presented. The results are of great interest because the student is able to find appropriate experimental conditions to esterify bilirubin and separate the products by HPLC. Likewise, different yields of the reaction are found depending on the choice of the aliphatic alcohol, being the more reactive methanol. A search for better conditions to increase the yield of the esterification of bilirubin with *n*-alcohols containing a larger number of carbons should be pursued.

To my opinion the thesis fulfils all requirements for bachelor thesis and I propose to grade it as EXCELLENT.

Finally, the student should make the effort to clarify some questions that remain unclear after my reading.

1) The student states that under certain experimental conditions (for example, longer incubation time) more isomers were formed. How does the student conclude this? Could it be possible that other (degradation) products were formed?
2) No absorption spectra of the HPLC peaks are shown to distinguish between different products or isomers with different retention.
3) The largest yield of the esterification of bilirubin is found with methanol. Has the student investigated if this can be an advantage and to use methyl-bilirubin in trans-esterification reactions to increase the yield of the esters of bilirubin with alcohols containing a larger number of carbons?

In České Budějovice on the 7th of September 2012

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